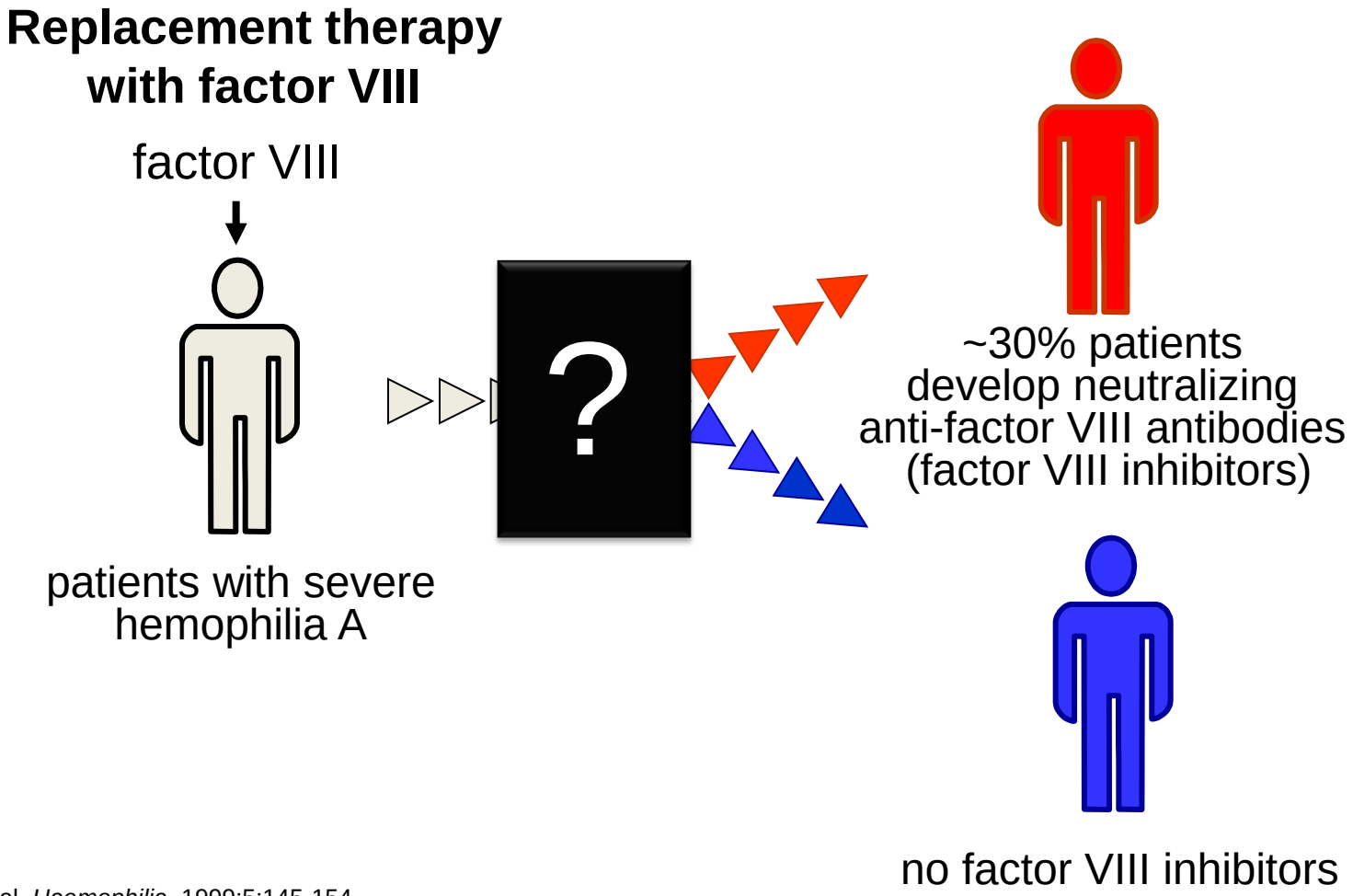
Case study: Specification of CD4+ T cell epitopes of human FVIII

Birgit Reipert
Director Immunology
TA Hemophilia/Hematology
Baxter BioScience

Hemophilia A

- X-linked recessive bleeding disorder that affects 1 in 5,000-10,000 men
- caused by mutations in the gene that codes for human clotting factor VIII
- gene mutations lead to either diminished function of factor VIII or lack of endogenous production of factor VIII
- Clinic: spontanous bleedings and hemorrhages

Major complication of replacement therapy in hemophilia A

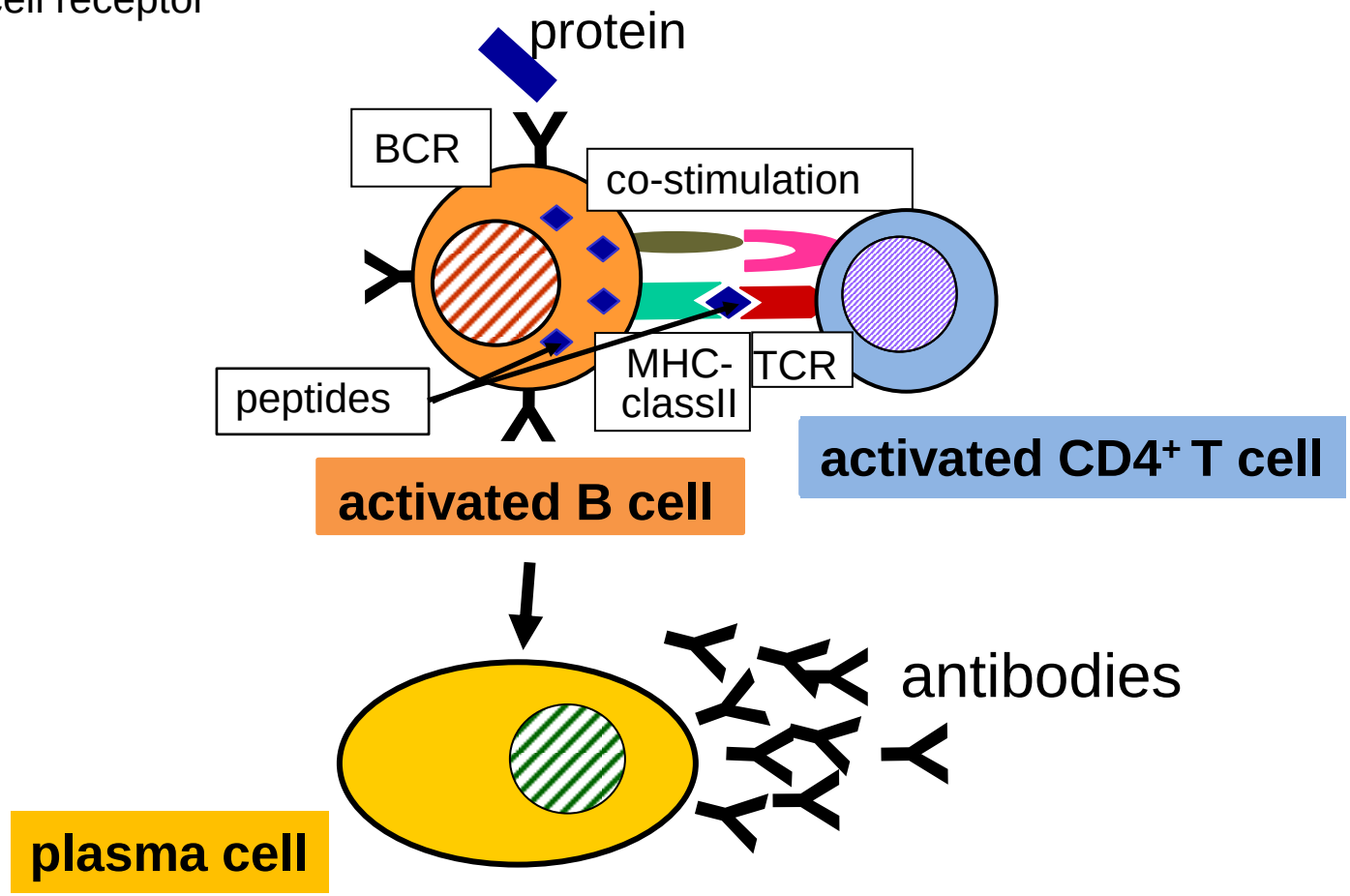


Scharrer I et al. *Haemophilia.* 1999;5:145-154.

T-cell dependent antibody responses against proteins

BCR: B cell receptor

TCR: T cell receptor



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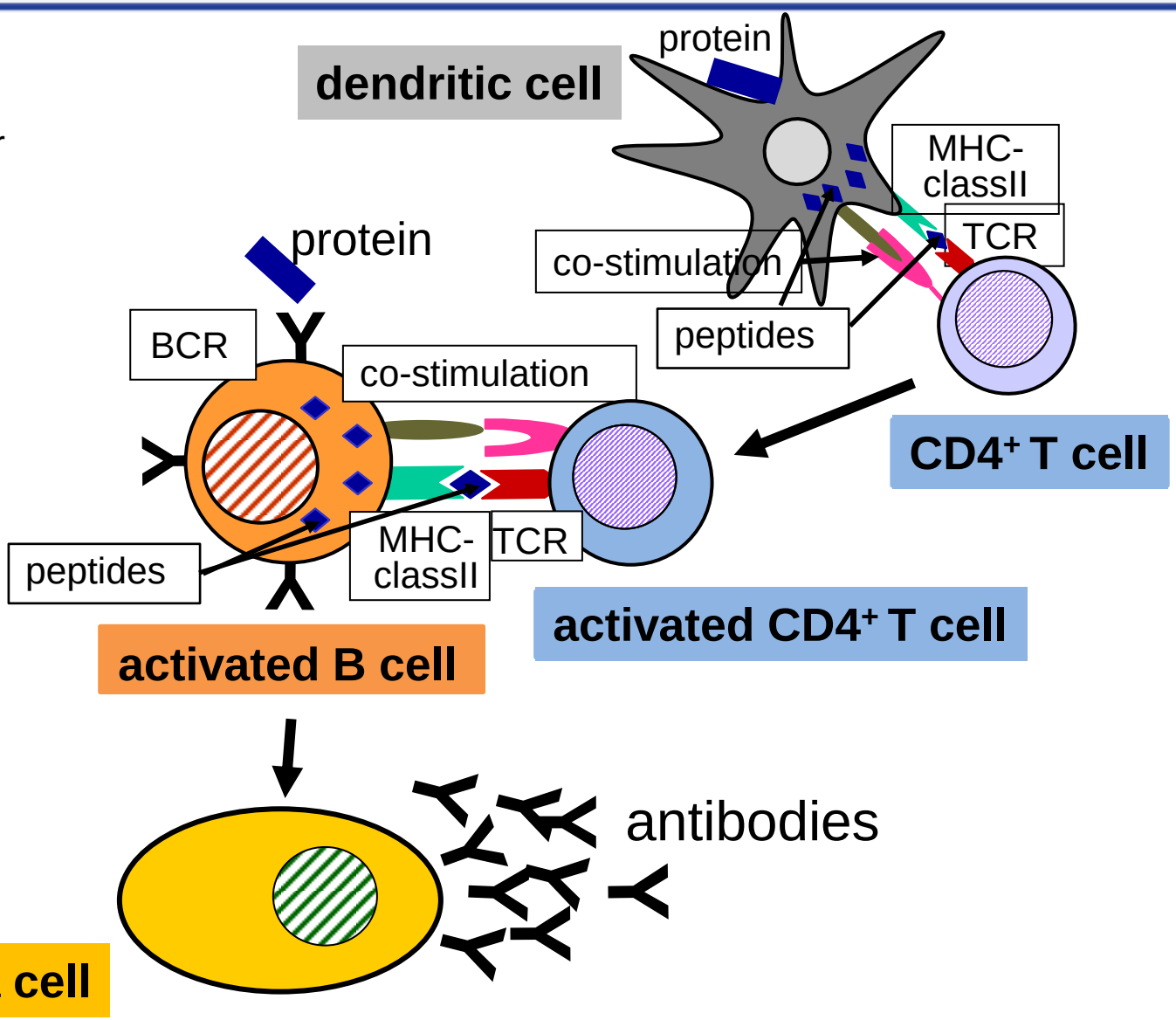

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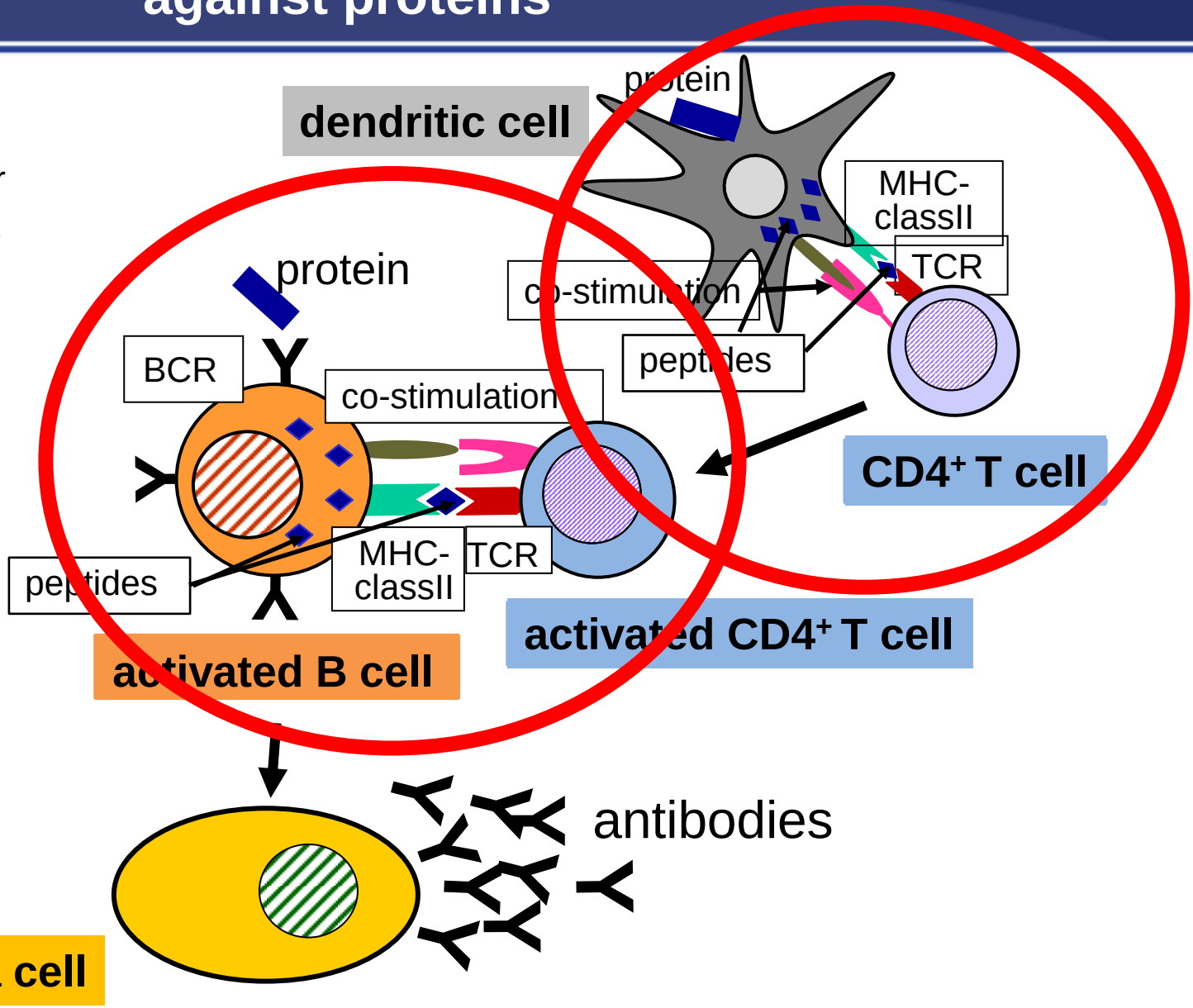
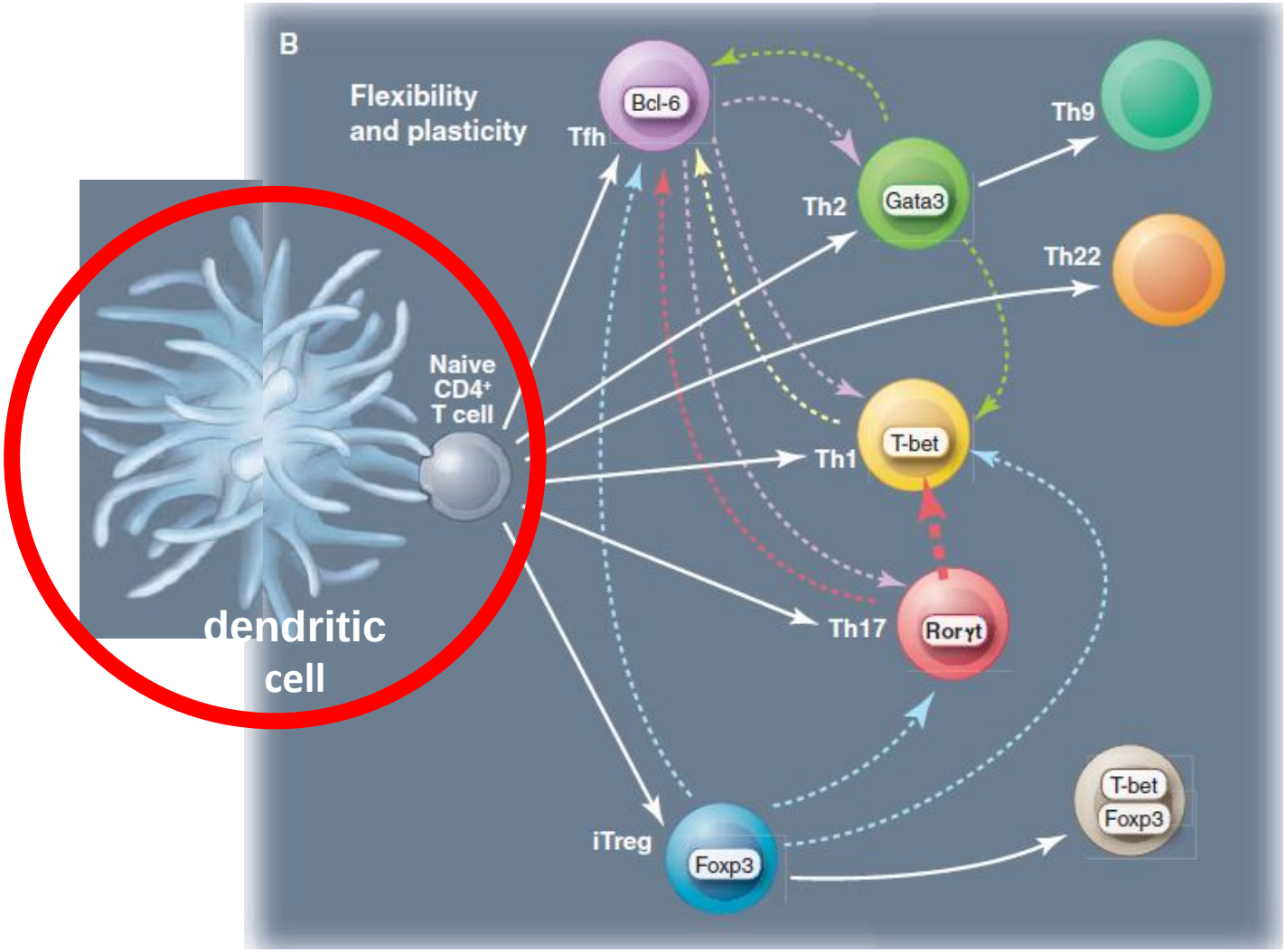


Flexibility and plasticity of peripheral CD4+ T cells



O Shea et al. *Science.* 2010; 327(5969):1098-1102.

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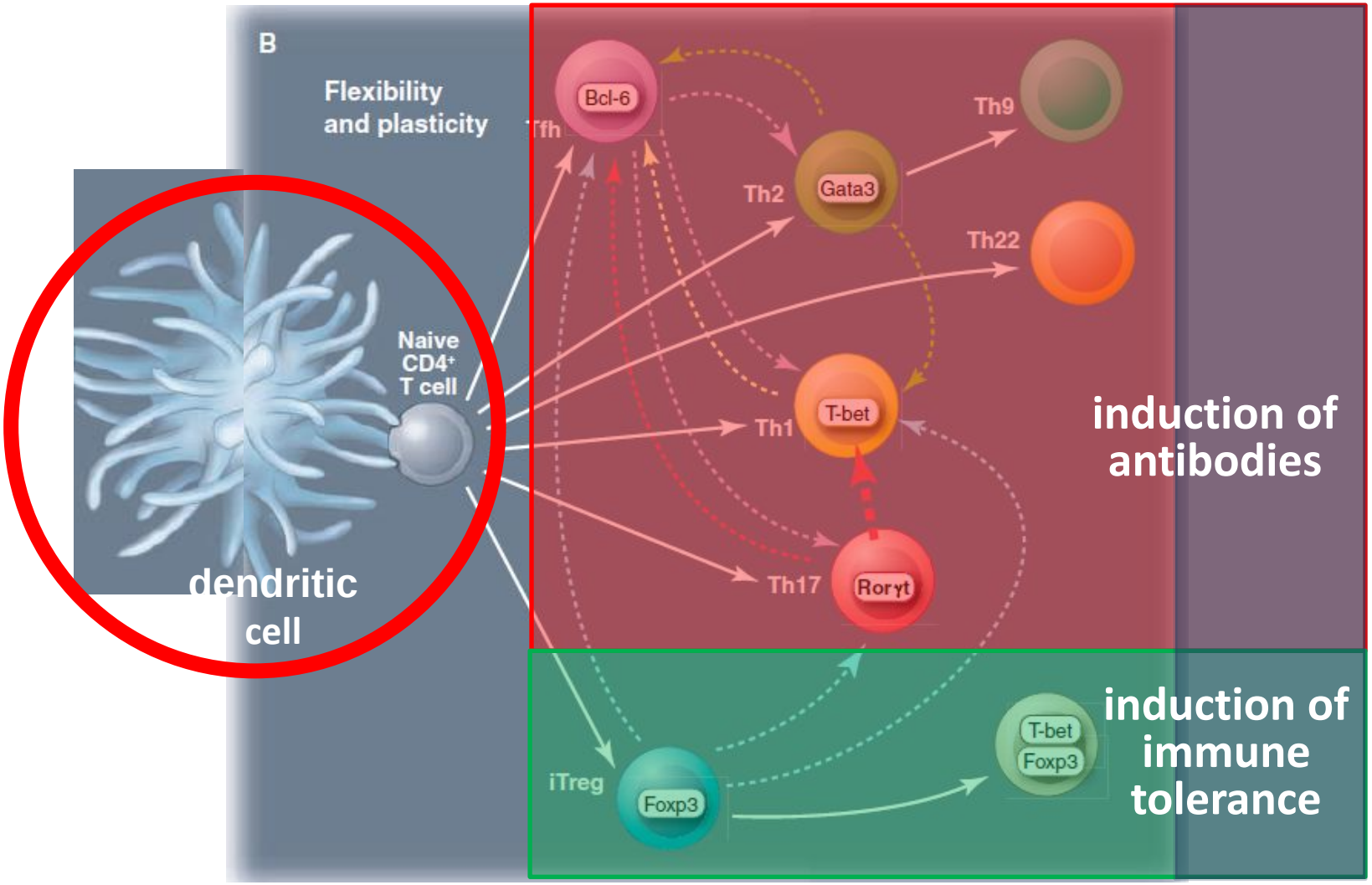

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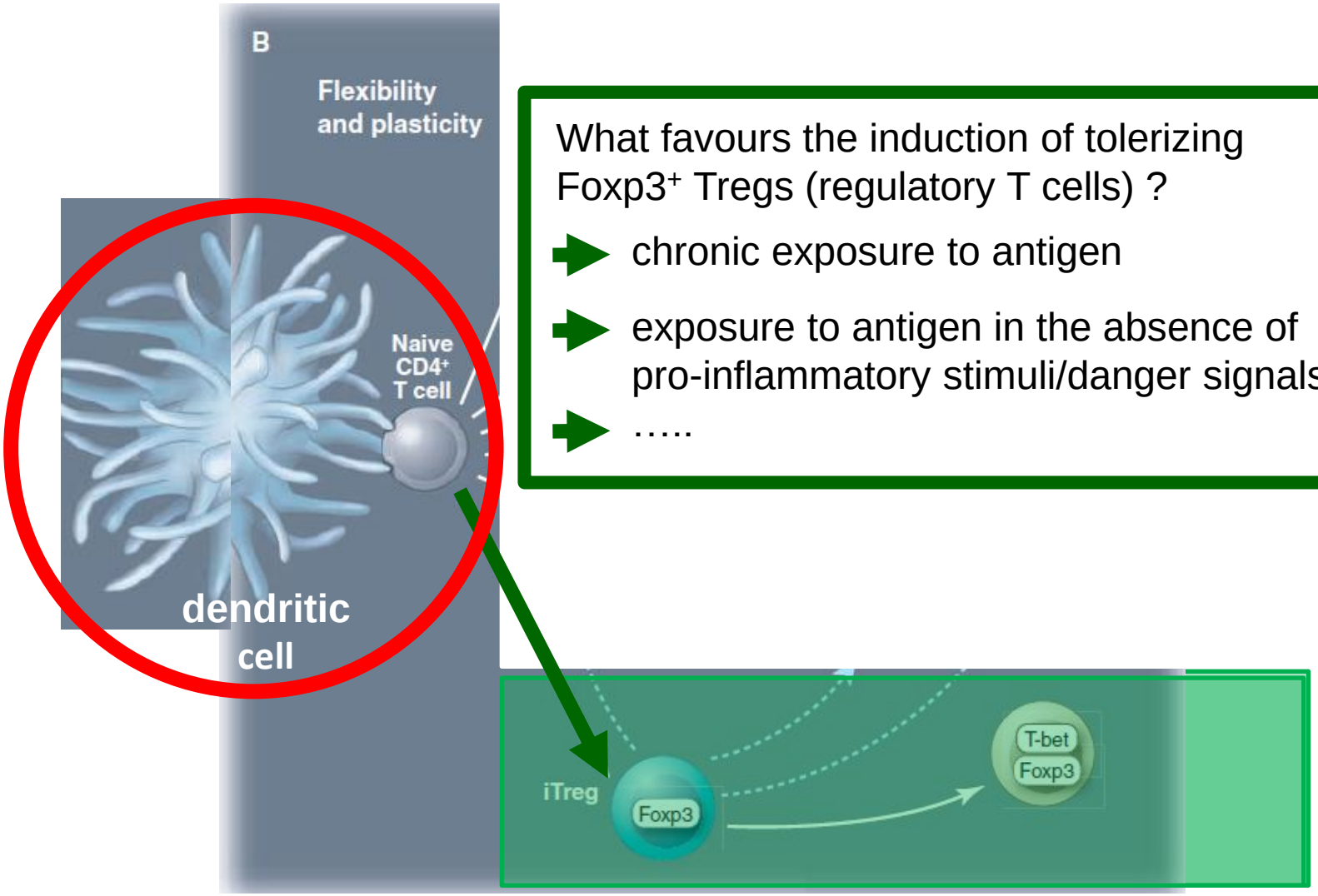
Foxp3+ CD4+ T cells favour induction of immune tolerance



What favours the induction of tolerizing Foxp3+ Tregs (regulatory T cells) ?

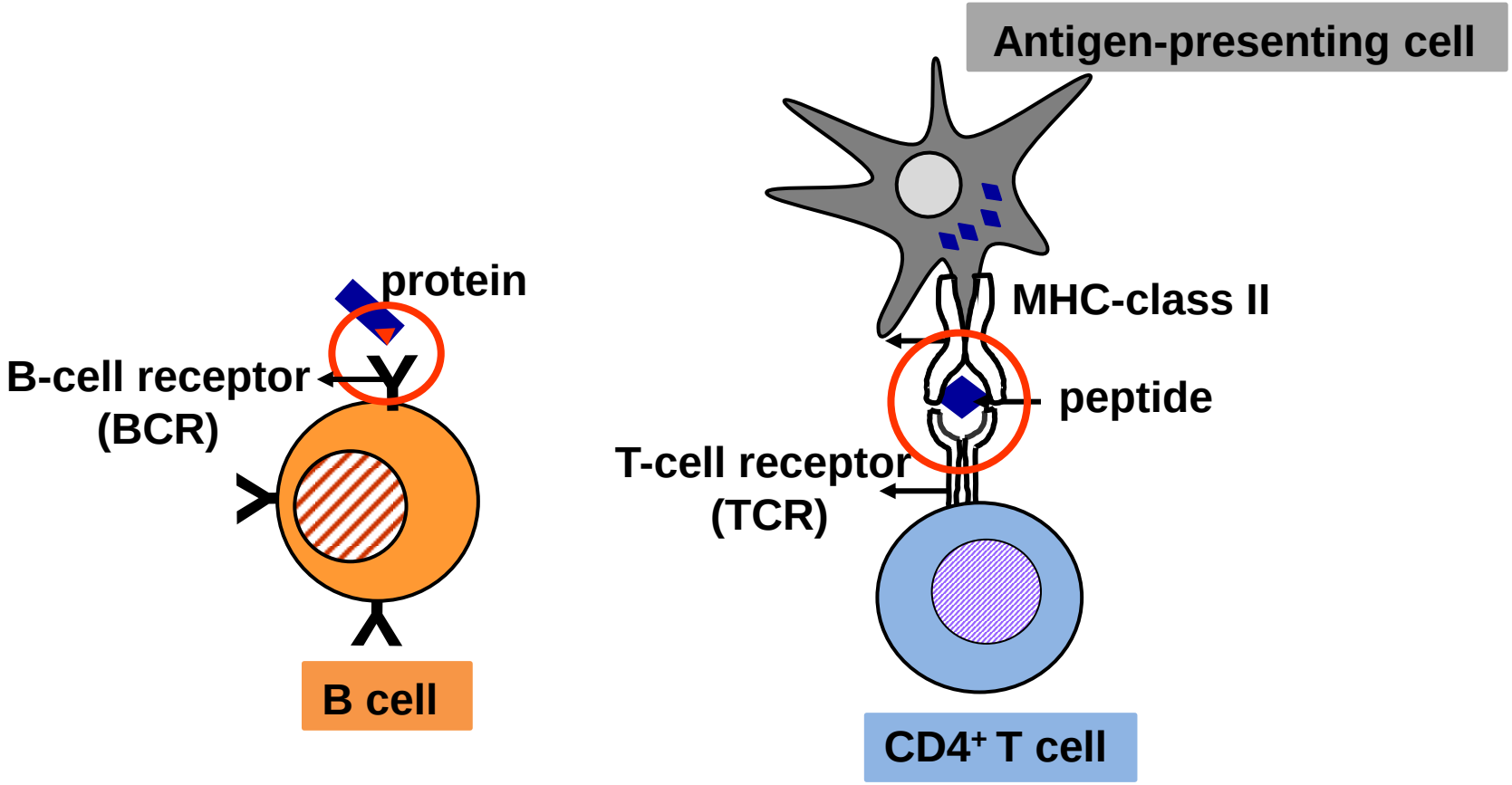
- chronic exposure to antigen
- exposure to antigen in the absence of pro-inflammatory stimuli/danger signals
- …..

O Shea et al. *Science.* 2010; 327(5969):1098-1102.

Specification of $CD4^+$ T cell epitopes of human factor VIII

T-cell dependent antibody responses against proteins

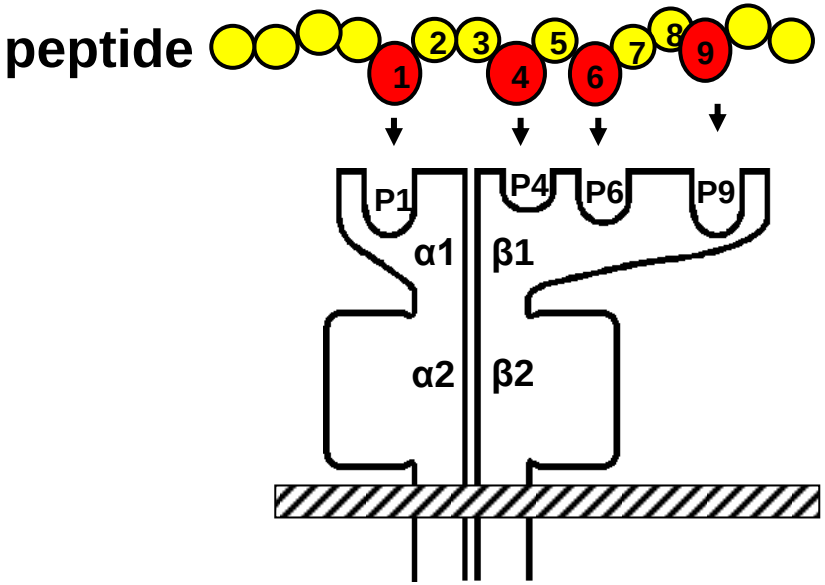
B cells and T cells recognize proteins in different ways



Peptides presented by MHC-class II

T-cell receptor contact residues

MHC-class II anchor residues of the peptide



MHC-class II molecule

Specification of CD4+ T cell epitopes of human factor VIII

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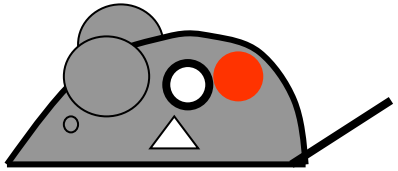
Our Approaches:

1. **Humanized hemophilic mice**
2. **In vitro binding assays using the Pro Immune – REVEAL™ MHC peptide binding assay**

Partially humanized hemophilic mice for identification of major factor VIII T-cell epitopes



E17 hemophilia A mouse
(kockout of the murine *factor VIII* gene -△)
that carries human HLA-DRB1*1501 (●) and does not express any murine MHC-class II (◯)

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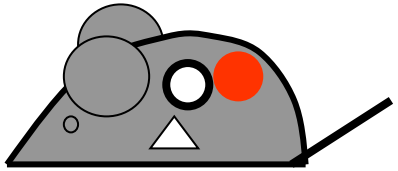

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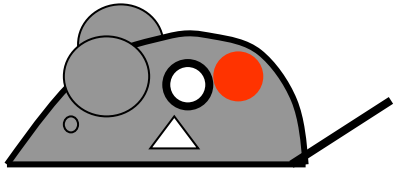

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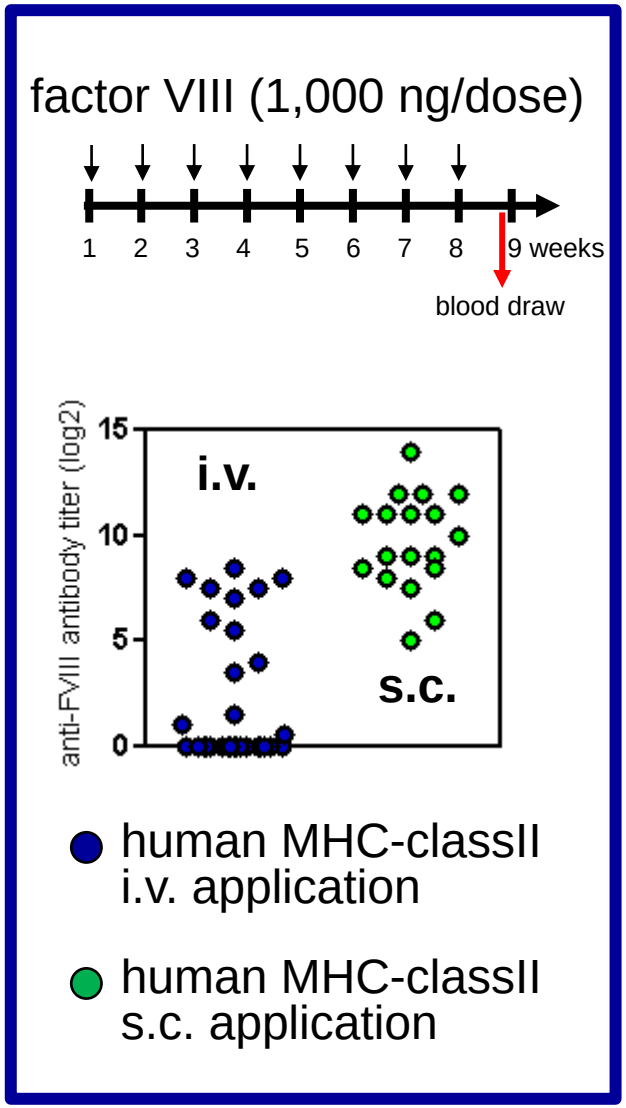
(kockout of the murine *factor VIII* gene -△)
that carries human HLA-DRB1*1501 (●) and does not express any murine MHC-class II (◯)

- Induction of antibody response against proteins depends on the presentation of immunogenic peptides by the human HLA-DRB1*1501

- The MHC-class II haplotype HLA-DRB1*1501 is associated with an increased risk for patients to develop neutralizing antibodies against factor VIII (Oldenburg et al. 1997; Pavlova et al. 2009)

CD4+ T-cell responses specific for factor VIII



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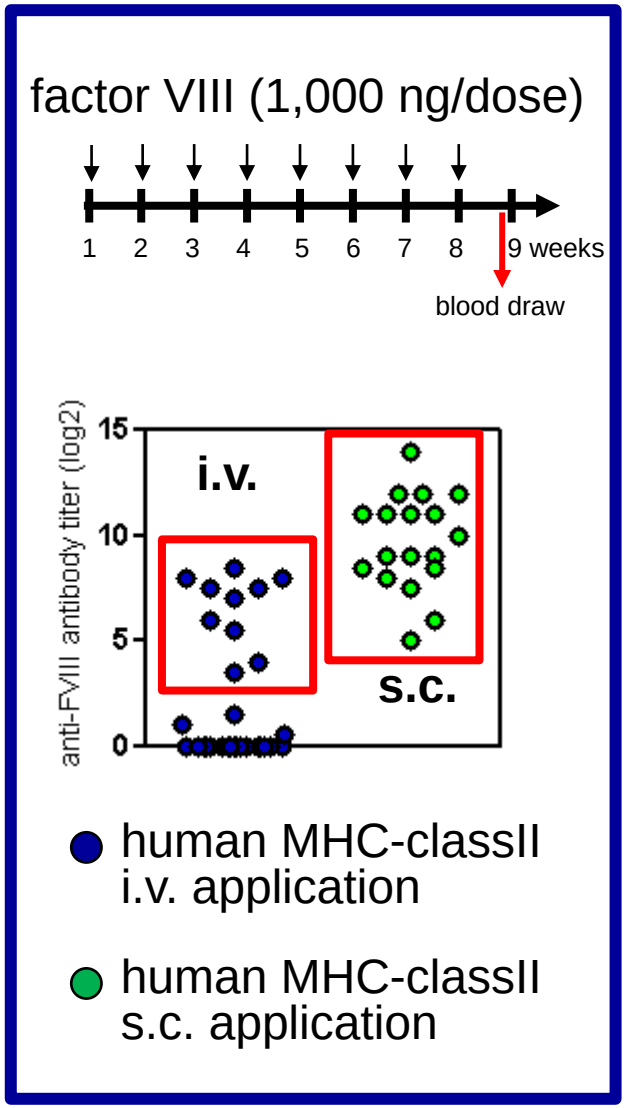
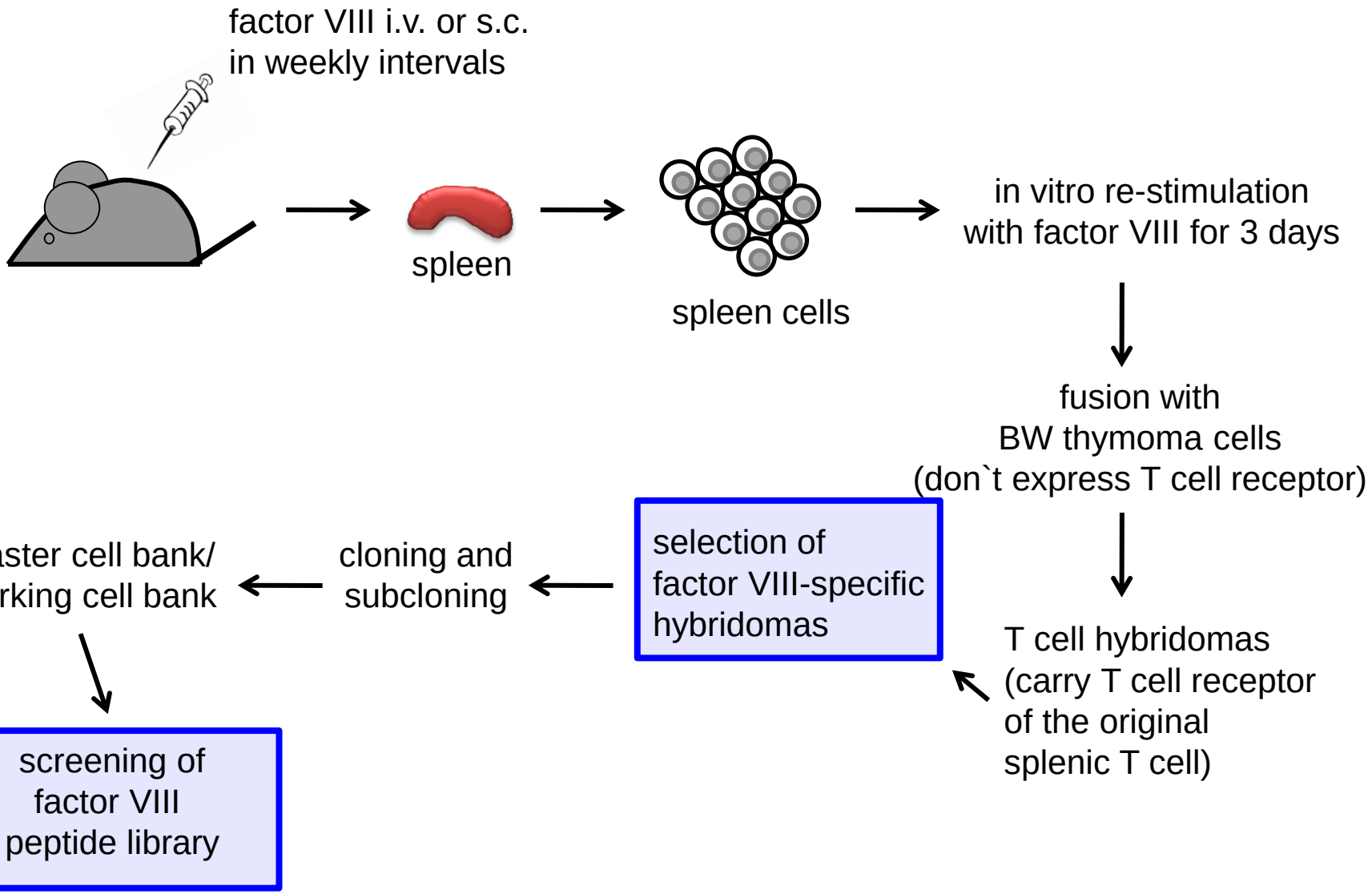


What are the major factor VIII T-cell epitopes (factor VIII peptides presented by MHC-classII) that drive immune response after i.v. and after s.c. factor VIII ?

CD4+ T-cell responses specific for factor VIII



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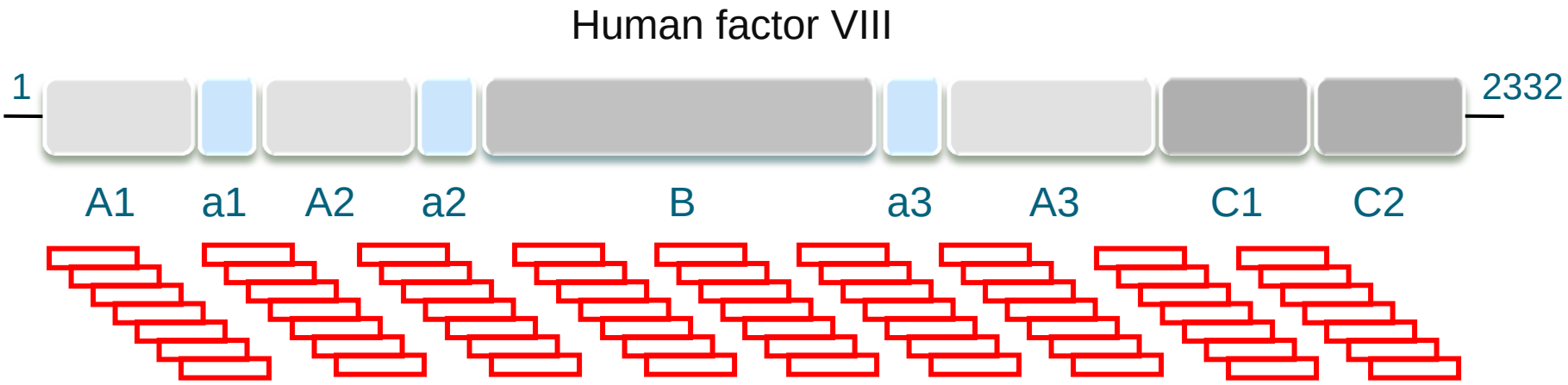
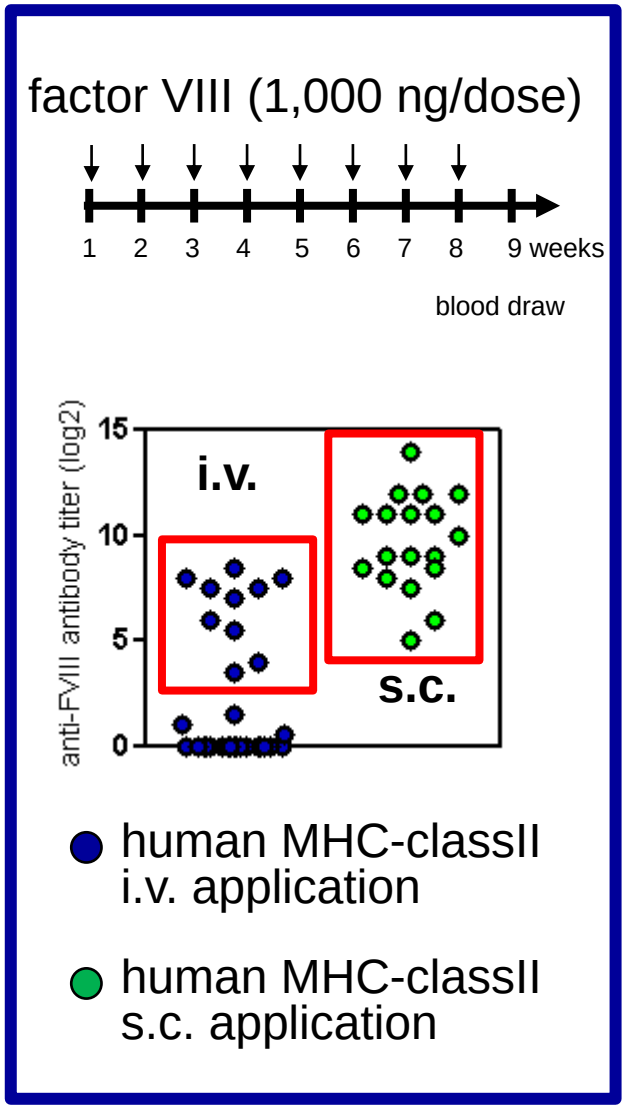


peptide library (15 mers with 3 AA offset)

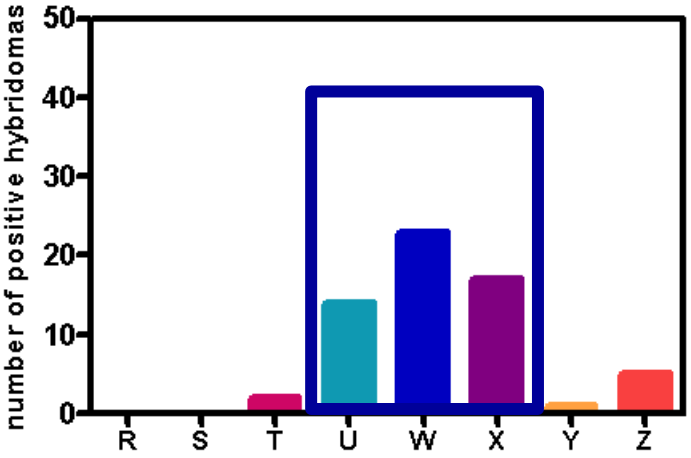
780 different peptides consisting of 15 amino acids each were tested in pools

Each T-cell hybridoma was tested with the whole peptide library
to specify the T-cell epitope that is recognized

CD4+ T-cell responses specific for factor VIII



i.v. factor VIII - 62 hybridomas tested so far



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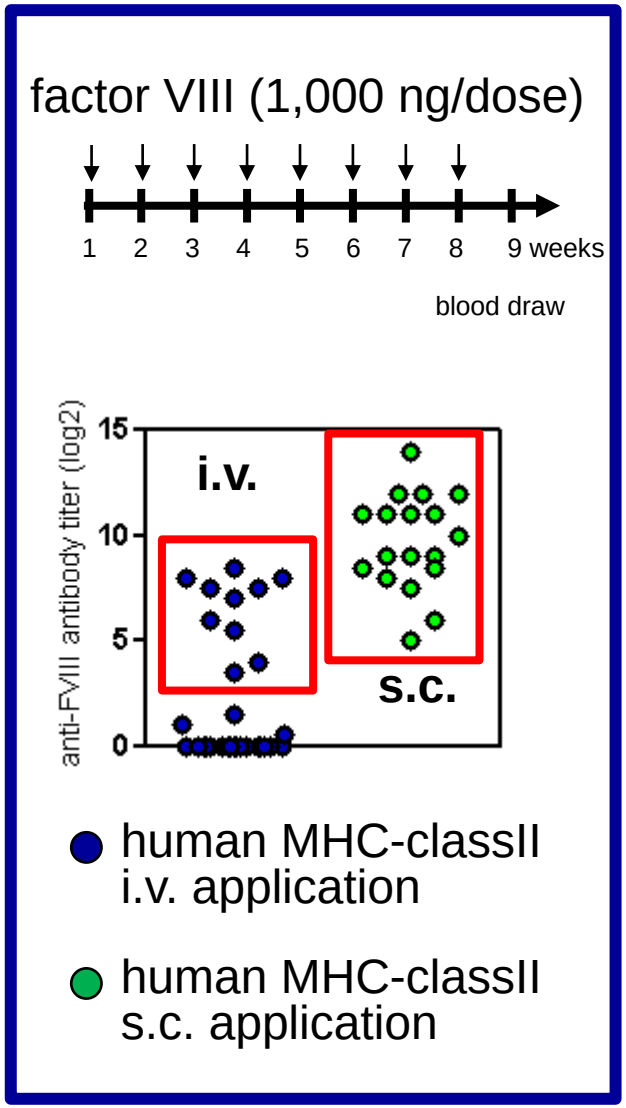

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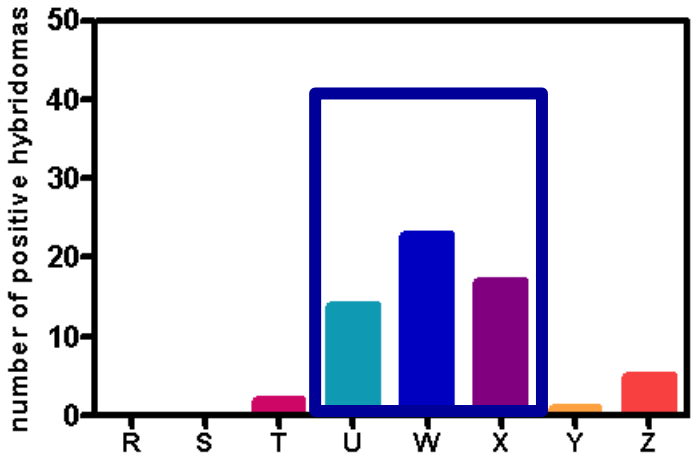
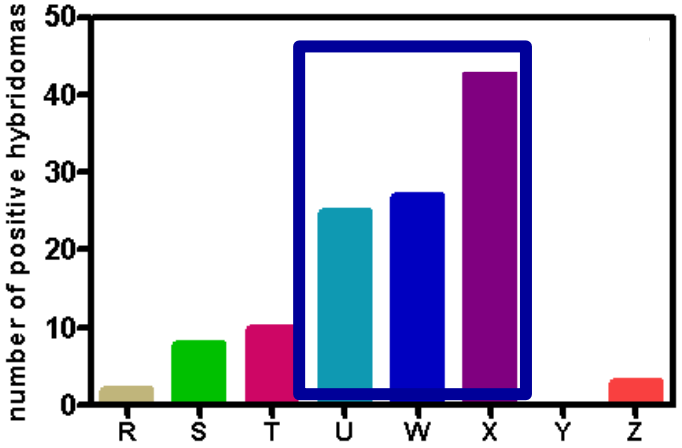
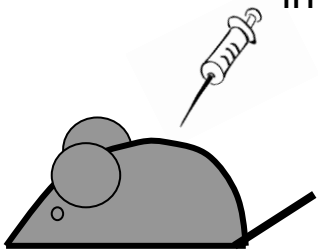


s.c. factor VIII - 118 hybridomas tested so far

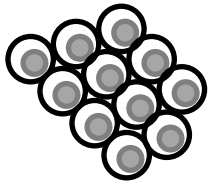


Validation of T-cell epitopes

factor VIII i.v. or s.c.
in weekly intervals



spleen



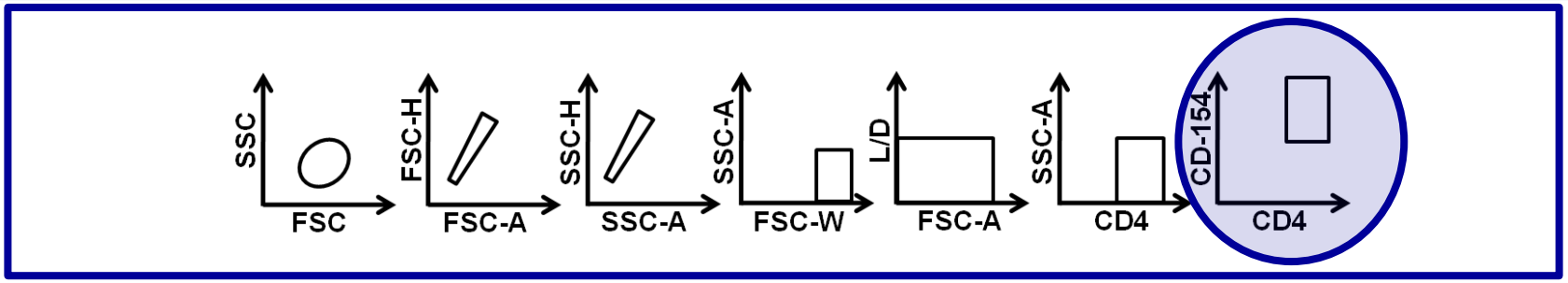
spleen cells

restimulation with
factor VIII or peptides
for 6 hours

FACS analysis of
activated $CD4^+$ T cells
after staining of spleen cells with:

- live/dead cell marker
- anti-CD4 antibody
- anti-CD154 (CD40L) antibody
  (activation marker, intracellular staining)

Validation of T-cell epitopes



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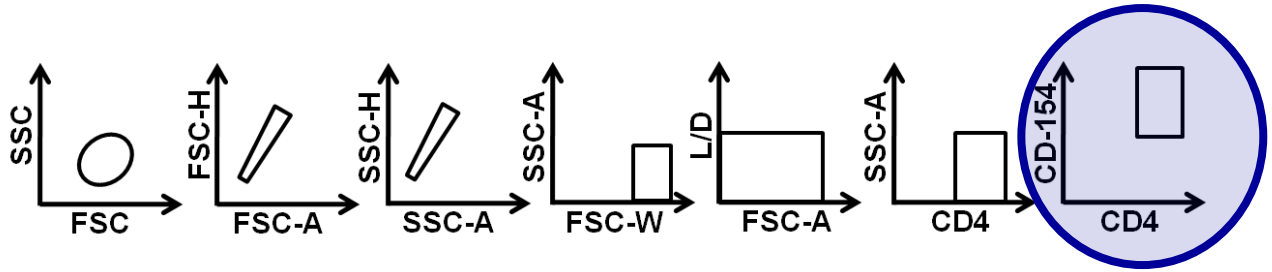

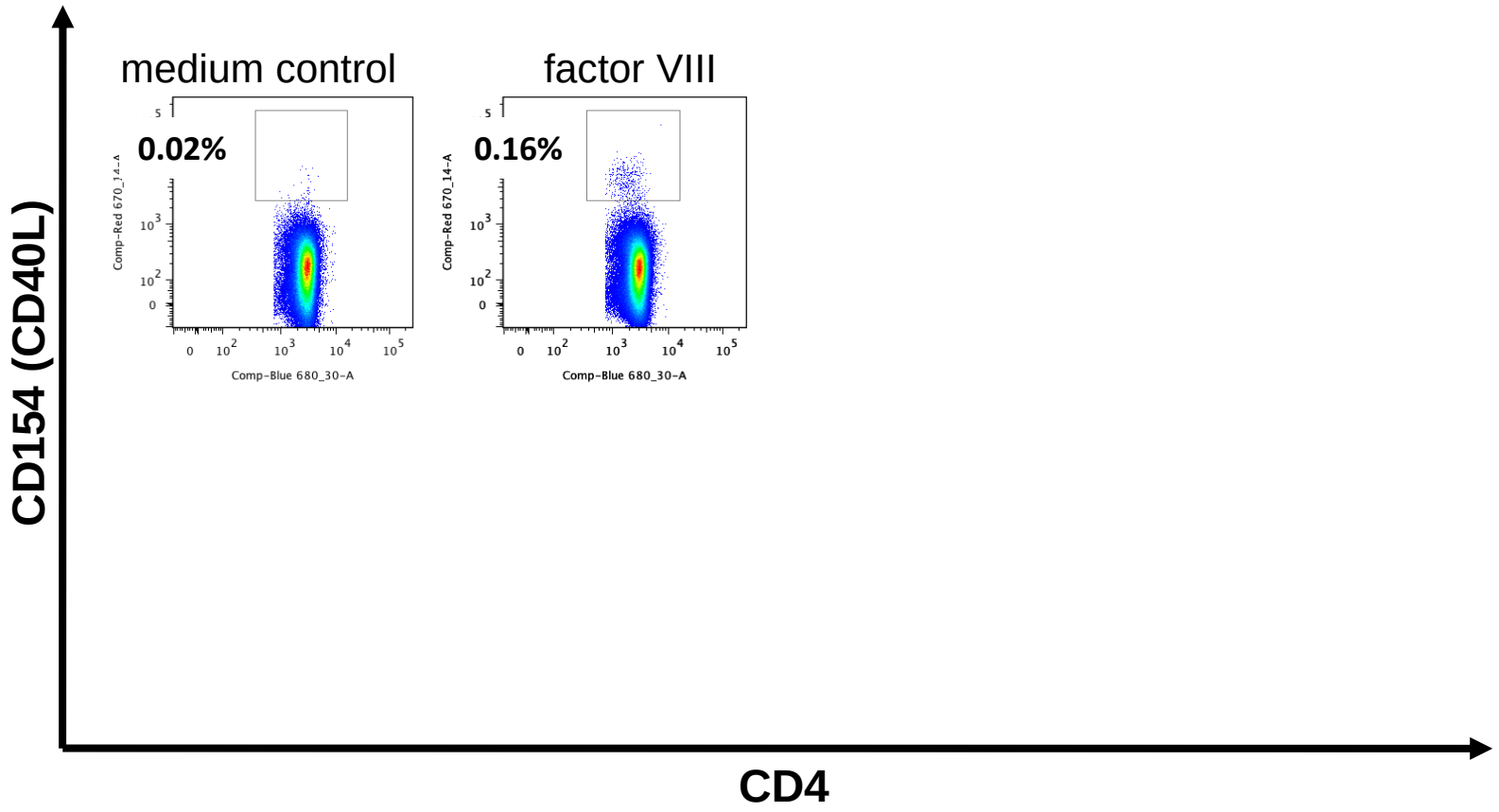

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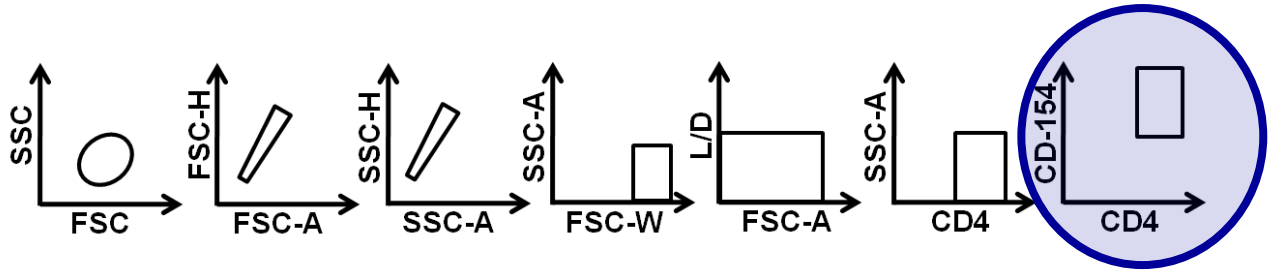

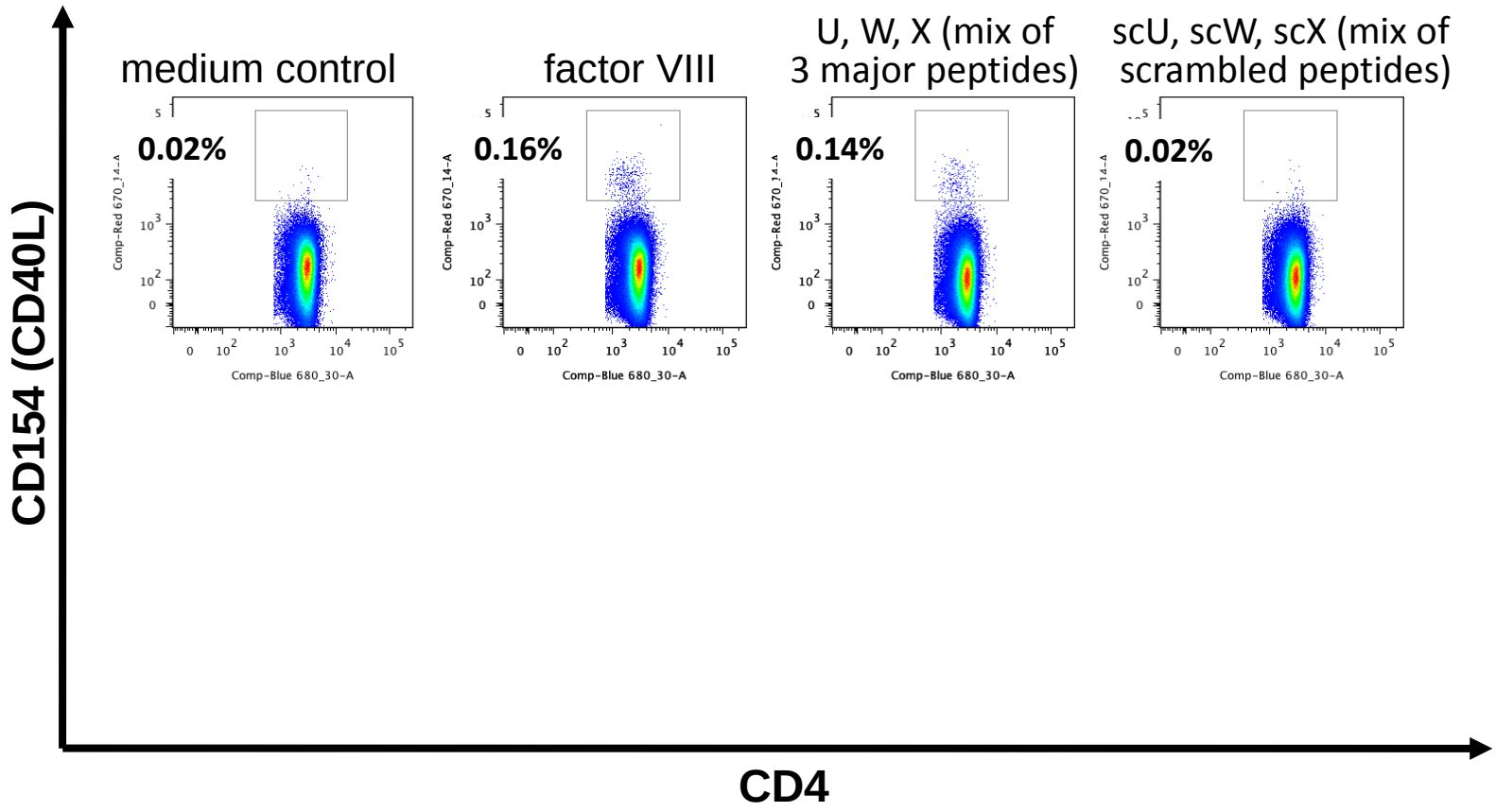

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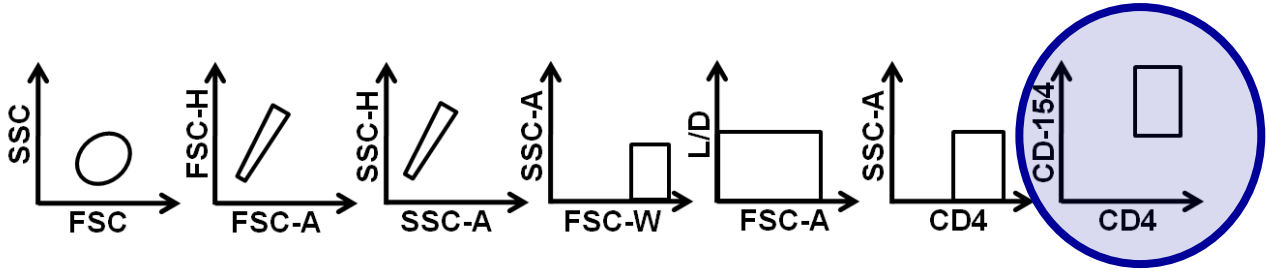
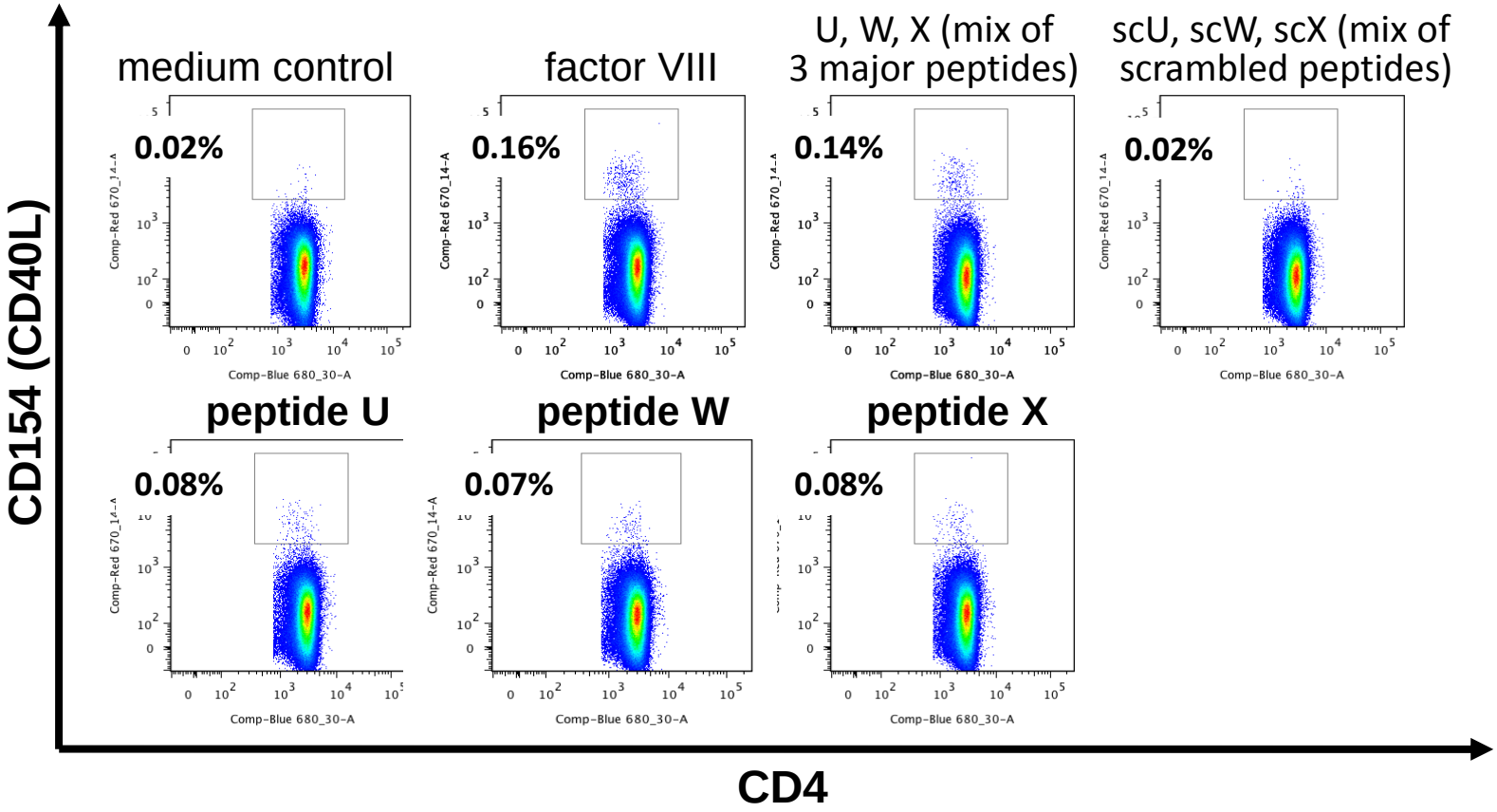
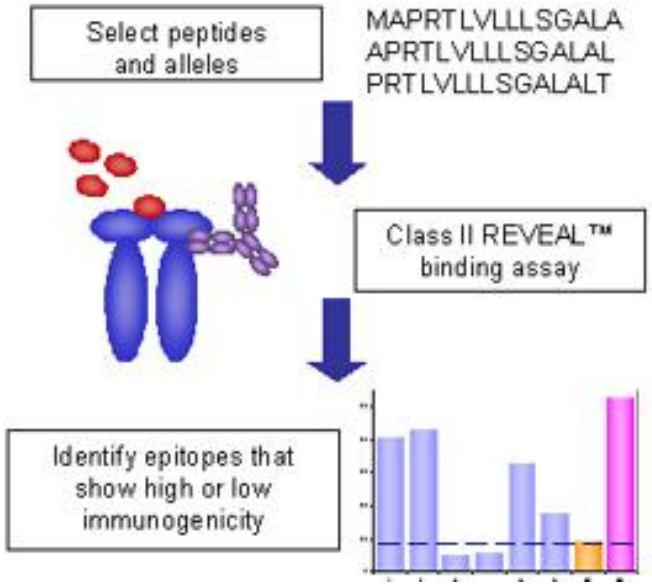




Pro Immune – REVEAL™ MHC peptide binding assay

PROIMMUNE
www.proimmune.com



The assay determines the ability of each candidate peptide to bind to
a MHC-class II protein of choice compared to a positive and an intermediate control peptide

The assay is measuring the ability of each peptide to stabilize the MHC-peptide complex.

Detection is based on the presence or absence of the native conformation of this MHC-peptide complex.

Binding of factor VIII peptides to multiple MHC-class II haplotypes

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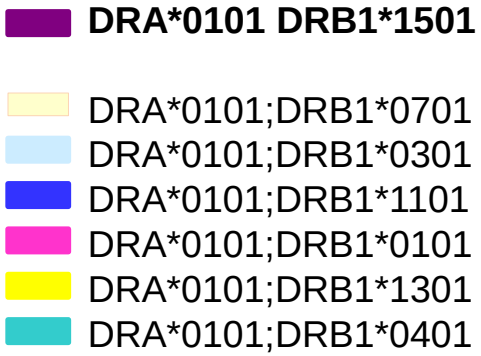
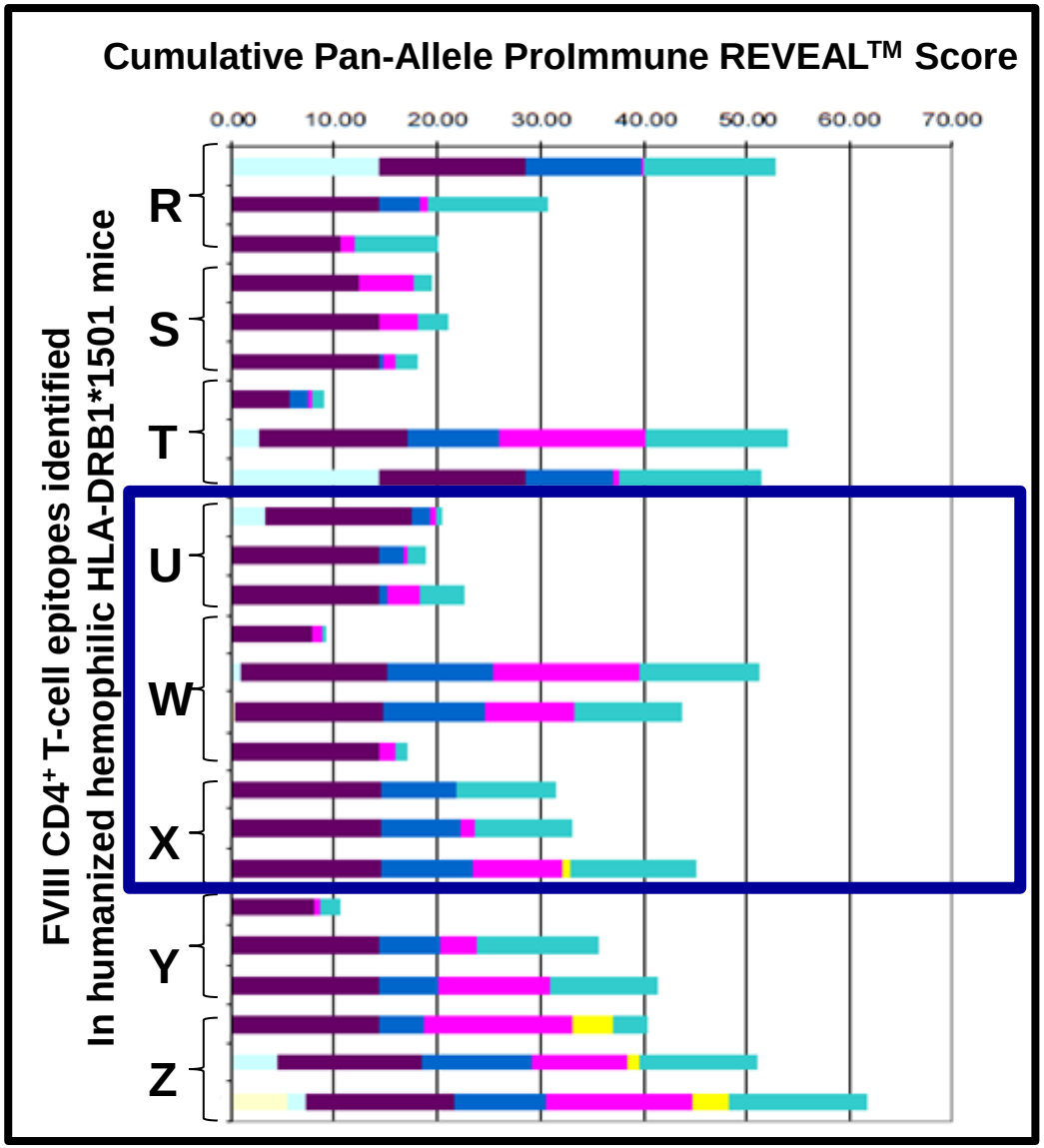


	US	Europe	World
DRA*0101 DRB1*1501	**10.6%**	**11.5%**	**6.2%**
DRA*0101;DRB1*0701	11.8%	11.6%	8.3%
DRA*0101;DRB1*0301	10.2%	15.3%	6.5%
DRA*0101;DRB1*1101	5.6%	6.1%	4.1%
DRA*0101;DRB1*0101	7.0%	7.5%	3.1%
DRA*0101;DRB1*1301	5.7%	6.3%	3.1%
DRA*0101;DRB1*0401	6.6%	6.4%	1.7%

Most of the factor VIII peptides identified bind to multiple MHC-class II haplotypes



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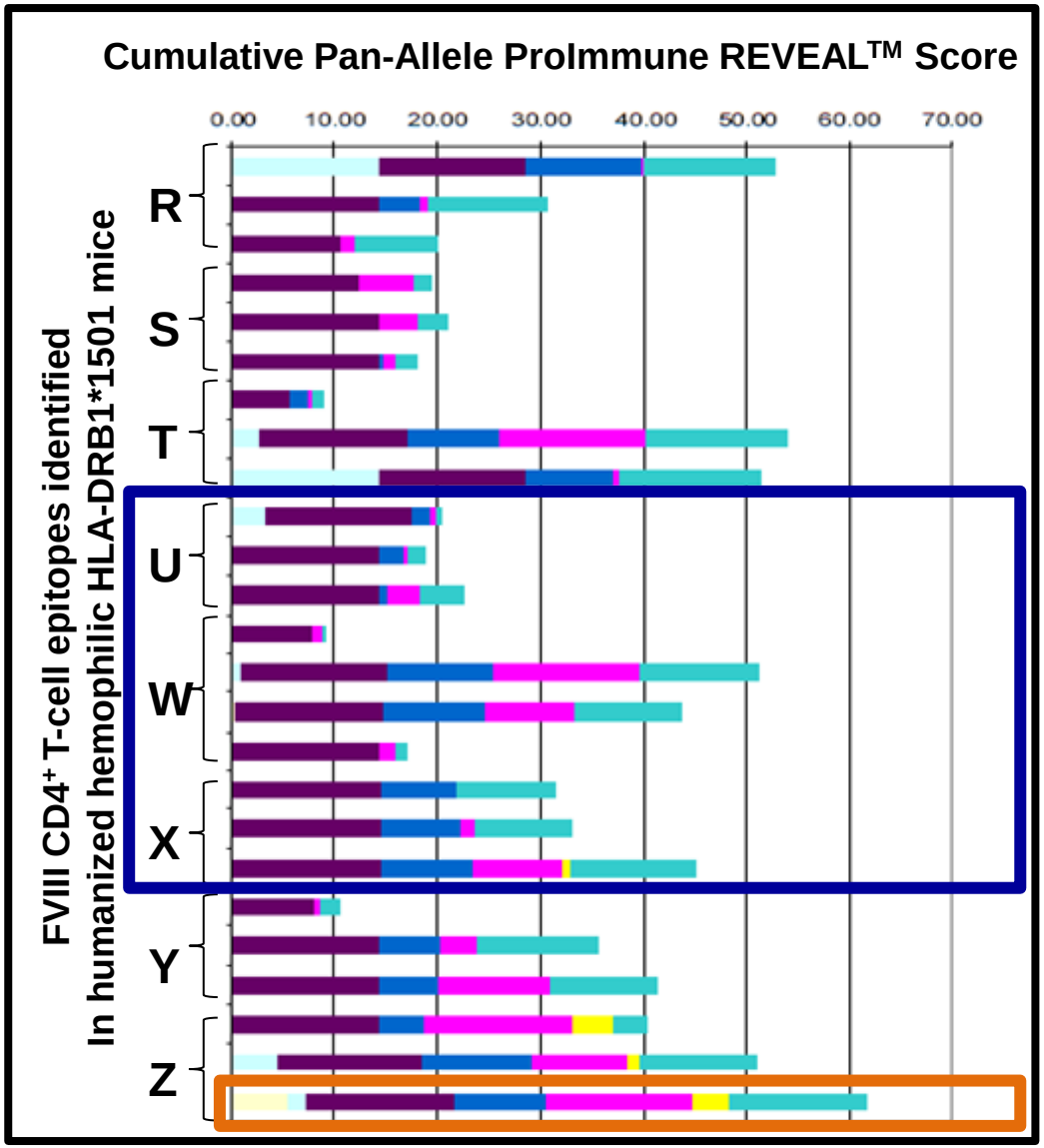

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Conclusion

- Antibody responses against factor VIII in humanized hemophilic HLA-DRB1*1501 mice depend on the application route.
  The incidence of antibodies is higher after s.c. than after i.v. application.

- A limited set of factor VIII peptides ($CD4^+$ T-cell epitopes) drive anti-factor VIII immune responses in humanized hemophilic HLA-DRB1*1501 mice.
  Immunodominant factor VIII peptides are the same after i.v. and s.c. application of factor VIII.

- Factor VIII-specific $CD4^+$ T cell epitopes identified in humanized hemophilic HLA-DRB1*1501 mice bind to a number of different HLA-DRB1* haplotypes when tested with ProImmune`s REVEAL class II technology.
  These results indicate that most of the factor VIII epitopes identified are promiscuous epitopes.

Acknowledgement

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PhD student
Baxter BioScience

Baxter BioScience

Lars Fugger
Oxford University
UK

Brigitte Binder
Research Associate
Baxter BioScience

ZIT

Center for Innovation and Technology
City of Vienna, Austria

PROIMMUNE
www.proimmune.com

David Wraith
Bristol University
UK

Thank you
for your attention

Antibody responses against FVIII

8 i.v. doses of FVIII (1000ng), given in weekly intervals